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FOREWORD

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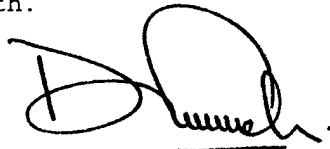
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Principal Investigator's Signature

07 Jan. 1998

Date

**COOPERATIVE AGREEMENT DAMD17-96-2-6001
MALARIA ECOLOGY, TRANSMISSION, IMMUNOLOGY,
PARASITOLOGY AND PROPHYLAXIS IN KENYA**

1996 & 1997 ANNUAL REPORTS

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INTRODUCTION

Over the past 25 years, the U.S. Army and the Kenya Medical Research Institute (KEMRI) have participated jointly in medical research projects. Although the scope of the work has changed from an initial interest in trypanosomiasis to current efforts which focus almost exclusively on malaria research, the collaboration has remained strong and has resulted in significant advances for the understanding and control of tropical diseases in Kenya and East Africa in general. This report summarizes the collaborative effort for calendar years 1996 and 1997.

Malaria is a potentially lethal parasitic infection of the blood which is spread by the bite of the female anopheline mosquito. Unprotected or non-immune persons who are bitten by an infective mosquito develop a febrile disease which can incapacitate and kill in a few days. Unfortunately, humans do not easily develop immunity to malaria infection and repeated illness is the rule. Treatment with drugs can effectively cure most infections, but the spread of drug resistance has made treatment and prevention much more difficult. Understanding the mosquito vector can better define the spread of malaria which, under the proper human and climatic conditions, can be truly epidemic. Kenyan people carry an enormous burden of malaria disease and western Kenya, where much of the work summarized in this report was conducted, is one of the most malarious regions of the world.

Studies conducted during 1996-97 under this Cooperative Agreement can be divided into the following broad categories: Malaria Immunology and Vaccine Development; Antimalarial Drug Studies; Malaria Vector Studies; and Other Tropical Disease Studies.

MALARIA IMMUNOLOGY and VACCINE DEVELOPMENT

Malaria remains one of the most militarily relevant infectious diseases effecting tropical troop deployments. KEMRI in collaboration with the US. Army Medical Research Unit-Kenya (USAMRU-K) has the unique ability to not only evaluate immune responses induced by natural exposure in individuals living in highly endemic regions of Kenya, but also to conduct studies in individuals immunized with potential malaria vaccine candidates. Other advantages of this collaborative effort are the close proximity of a well-equipped clinical and immunology laboratory facility to areas of high malaria transmission (typical travel time from field site to lab 1 hour) and the presence of individuals at KEMRI and USAMRU-K with the technical, clinical, and regulatory skills necessary to conduct basic and applied clinical research.

1996 AND 1997 ACCOMPLISHMENTS

A considerable amount of time and resources were expended beginning in mid-1996 and continuing throughout 1997 in the establishment of new field sites for both the malaria immunology and vaccine studies and the antimalarial drug testing project (to be discussed later). This was made necessary by the expansion of a large-scale antimalarial bednet study being conducted in the same general area in western Kenya by the U.S. Centers for Disease Control with a U.S. Agency for International Development funds. These new sites are described below. In addition to the new sites, some ongoing studies were also continued in established sites. All of the studies listed below are long-term in nature, following specific cohorts throughout 1996-97.

Isolation and Identification of Malaria

There is a continuing requirement to isolate, identify, and characterize malarial parasites in as many geographic areas as possible. Several major projects, were conducted jointly between KEMRI and USAMRU-Kenya in this area. These included: 1) A colorimetric assay for the diagnosis of Plasmodium infections. This test measures the activity of a parasite enzyme (parasite lactate dehydrogenase, or pLDH) in blood from infected individuals and may prove useful in the diagnosis of malaria in large populations. In addition, the pLDH test was evaluated as a non-radioisotopic method for measuring drug resistance among clinical isolates of Plasmodium falciparum malaria. 2) ELISA, Western blot, and immunofluorescence assays to measure auto antibodies against platelets and white blood cells in patients with malaria were further tested. Data suggest that a malaria parasite protein mimics host proteins and induces these autoantibodies. 3) Additional evidence was compiled, further demonstrating that elevated blood serum levels of

nitric oxide are correlated with disease severity in cerebral malaria and also disease resolution in visceral leishmaniasis (Kala Azar). 4) New isolates of P. falciparum malaria parasites from ongoing studies and additional areas of Kenya were added to the reference laboratory and assessed for the extent of anti-malarial drug resistance.

Characterization of Protective Immune Responses Against Malaria and of Structure, Function, and Antigenicity of Malaria Macromolecules

In order to produce effective vaccines against malaria, a thorough understanding of the basic immunology of the parasite is necessary. Again, this is often geographically dependent, and western Kenya has some of the highest rates of malaria transmission in the world. In both 1996 and 1997, malaria immunology again was a primary focus of research activities for KEMRI and USAMRU-K personnel at the field Laboratory in Kisian, Nyanza Province. Studies continued to concentrate on developing an understanding of how the malaria parasite binds to the wall of blood vessels and on the identification of compounds which inhibit this process. This work has shown that nearly all malaria parasites from non-pregnant donors in Western Kenya use the protein Sequestrin to bind to the CD36 in the blood vessel wall. Few individuals develop antibodies which are able to block this binding. Additionally, individuals naturally exposed to malaria appear to develop T cell, but not humoral, immunity to transmission-blocking proteins raising the possibility that malaria infection may boost immune responses to a transmission blocking vaccine. Observations from these studies will greatly simplify the development of such a vaccine.

For the past two years, during each rainy season in western Kenya (2 seasons per year), males between the ages of 12 and 35 have been treated to eradicate malaria parasites. They are then followed with weekly bloodsmears to determine susceptibility to re-infection. Samples are periodically obtained to measure immunologic recognition of malaria antigens, and the measurements were analyzed to determine associations with resistance to malaria. These studies, which are currently ongoing, have shown that (a) antigenic variation of the circumsporozoite protein (CSP) influences immunologic recognition, (b) cellular immune recognition of CSP correlates with susceptibility to infection, (c) the binding protein, sequestrin, is recognized widely among naturally exposed individuals. These immunologic observations can now be used in the design of newer generation malaria vaccines.

Working with the nursing staff and officers of the New Nyanza Provincial Hospital in Kisumu, samples of placenta and blood were collected from women at the time of delivery. These samples were examined to describe the type of malaria parasites and immune responses which occur in the placenta. Clinical information

was obtained about the mother and her newborn to determine the health consequences of malaria. The work thus far has shown that a distinct subpopulation of Plasmodium falciparum parasites causes maternal malaria by binding to chondritic sulfate A on the wall of the placenta. Malaria infection in the placenta can cause immune cells to populate the tissue and release soluble mediators of inflammation. These soluble mediators (cytokines) alter the physiologic immune environment of the placenta, and possibly interfere with the development of the fetus. The results from this study have been widely recognized as a major breakthrough in the understanding of maternal malaria. Laboratory work is now being extended to identify compounds which might prevent the parasite from binding to the wall of the placenta and also to understand the effect of the placental immune response on the fetus. Additional samples were obtained from the District Hospital in Kericho, an area of 'highland' malaria to determine the differences in parasite and host responses between the two endemic areas in western Kenya.

Malaria Vaccine and Vaccine Study Site Development

Clinical trials of the malaria vaccine known as Spf66 have been in progress in various parts of the world. Because subcutaneous administration of this vaccine can frequently cause local reactions, a study was completed in western Kenya in 1996 to determine whether delivery of the vaccine by intramuscular route can reduce these reactions without impairing the immune response. The study demonstrated that intramuscular administration of the vaccine significantly reduced the frequency of local reactions such as pain, redness, and swelling at the site of injection. However, measures of cellular immune responses (lymphocyte proliferation, cellular release of gamma interferon) were lower among intramuscularly-inoculated vaccinees as opposed to those inoculated subcutaneously. Malaria infections were studied as secondary endpoints for analysis, and vaccination with Spf66 did not have a measurable impact on frequency or levels of parasitemia among these semi-immune adults. PCR techniques were developed to determine whether specific parasite strains targeted by the vaccine were eliminated from the study population. Parasite strains targeted by the vaccine occurred with equal frequency among recipients of Spf66 and recipients of Energix B, the placebo.

As stated above, a considerable amount of effort was expended in establishing a new field site for the testing of a new malaria sporozoite vaccine, RTS,S, which is currently under development at the Walter Reed Army Institute of Research. An area centered on the village of Kombewa, Nyanza Province, was selected because of its similarity to previous successful study sites and its proximity

to the Kisian central laboratory. A suitable population of potential volunteers for a vaccine study was identified and baseline immunological studies of this population were initiated to characterize the malaria infection in this area. These studies will continue, with clinical testing of the new vaccine targeted for late 1998.

ANTIMALARIAL DRUG STUDIES

Antimalarial drug studies in Kenya have focused on malaria chemoprophylaxis, i.e., the prevention of the clinical disease caused by malaria infection through the use of antimalarial drugs. Most currently used drugs such as doxycycline and mefloquine prevent or suppress disease development, but not infection by the parasite from the mosquito. There are several problems with malaria chemoprophylaxis which require further research: drug resistance, poor compliance, drug side-effects. Malaria drug resistance is a growing problem which causes new drugs to become ineffective within 5-10 years of their introduction through the evolutionary selection of parasites able to survive drug treatment. Chloroquine is currently ineffective in Africa, pyrimethamine/sulfadoxine is rapidly failing, and there are reports of mefloquine prophylaxis failures. Drug compliance directly relates to the number of times drug administration is required (i.e. daily or weekly) and the perceived risk/benefit of the drug from the patient's point of view. Drug side-effects are inevitable when potent drugs are given to large numbers of healthy people. Nothing discourages compliance with a prophylaxis regimen as much as the idea that a certain drug causes unpleasant side-effects. The goal of current chemoprophylactic drug research is to discover new and better regimens to prevent malaria and Kenyan field sites play a key role in this program.

1996 AND 1997 ACCOMPLISHMENTS

During 1996, KEMRI/USAMRU-Kenya investigators, in collaboration with Glaxo Wellcome South Africa Ltd., conducted a randomized, double-blind, placebo-controlled, parallel group study to determine the chemosuppressive activity of a fixed dose combination of atovaquone/proguanil (Malarone^R) in patients at risk of developing P. falciparum malaria in Lwak location in western Kenya. Adult subjects (212) received an initial treatment course of four tablets of atovaquone/proguanil (250 mg/100mg per tablet) daily for 3 days to effect radical cure in parasitemic volunteers and to eliminate inapparent infection. Immediately thereafter, 196 of these individuals were randomly assigned to receive one of three prophylactic regimens: one atovaquone/proguanil tablet daily (n=67), two atovaquone/proguanil tablets daily (n=64), or placebo (n=65), for 10 weeks. Weekly malaria smears were taken, and the study endpoint for any single subject was the development of confirmed parasitemia on blood smear while receiving prophylaxis. Subjects were evaluable if they developed malaria or completed all 10 weeks of

prophylaxis. Of the evaluable subjects (90% of total enrollees), all in the low-dose (60/60) and high-dose (61/61) atovaquone/proguanil groups remained malaria-free during the 10-week prophylaxis period, in contrast to only 50% (28/56) in the placebo group ($P=0.001$). The atovaquone/proguanil regimens were as well tolerated as placebo. The most frequently reported drug-related adverse events, dyspepsia and gastritis, were reported in a similar number of patients in the 3 treatment groups. This study clearly demonstrated that daily atovaquone/proguanil is 100% efficacious for the chemosuppression of falciparum malaria in western Kenya. Glaxo Wellcome intends to file for licensure of Malarone^R with the U.S. Food and Drug Administration as both a malaria treatment and prophylactic. The KEMRI/USAMRU-K study will be included in the prophylaxis application as a pivotal study.

As stated previously, it was necessary to identify a new field site for continued testing of antimalarials in western Kenya. The remainder of 1996 and the first quarter of 1997 were focused on the development of a new site in Ndori, Nyanza Province. Like Kombewa, the vaccine site, Ndori is situated on a tarmac road and is easily accessible from the base laboratory in Kisian. A suitable building was leased and upgraded and a local population of volunteers was identified and recruited for the 1997 study.

The antimalarial drug study conducted in 1997 was the evaluation of weekly Etaquine (WR238605) compared to placebo for chemosuppression of P. falciparum in adult volunteers. Etaquine is a primaquine analog that is being developed by the U.S. Army and SmithKline Beecham pharmaceutical company as a potential chemosuppressive drug against both falciparum and relapsing malaria. Early clinical trials to date indicate that Etaquine is well tolerated in single doses up to 500 mg, it has a long half life of about 2-4 weeks making weekly administration practical, it is able to protect most volunteers against mosquito challenge with P. falciparum, and it is able to kill relapse forms of P. vivax in patients given simultaneous chloroquine. The study in western Kenya was designed to determine if Etaquine could be used in adult volunteers in order to prevent falciparum malaria in a highly endemic area. The recruitment of approximately 250 adult healthy residents of the Ndori area was conducted, as all human volunteer studies in Kenya are, by non-coercive means following written informed consent. Following a course to clear any pre-existing parasitemias, the volunteers were equally randomized into 4 groups: placebo, loading dose of Etaquine (500 mg daily for 3 days) followed by placebo, loading dose Etaquine followed by 250 mg weekly, loading dose Etaquine followed by 500mg weekly. Drugs and placebo were identical in appearance in order to maintain blinding. Drugs were given weekly under supervision by field workers, weekly malaria smears were taken, and monthly blood samples were taken for blood chemistry, hematology, and pharmacology analysis. The trial continued for 10-15 weeks of drug administration and a further 4 weeks of follow-up following

discontinuation of the medication. The study endpoint for any single volunteer was development of confirmed parasitemia on blood smear. The basic findings of this preliminary study were that persons with a normal Glucose 6 Phosphate Dehydrogenase (G6PD) weekly Etoquine is safe and well-tolerated. Etoquine given weekly was highly efficacious (greater than 95%) in preventing any malaria parasitemia in adult volunteers compared to a greater than 50% attack rate in the placebo group. At the conclusion of this study, preparations began immediately for a follow-on study to be conducted in 1998 in a different population in Bondo at a Teachers Training College approximately 12km west of Ndori. A protocol was prepared and entered into the approval process and a small satellite clinic was established in Bondo.

MALARIA EPIDEMIOLOGY

Malaria in the high altitude area of Kericho District, Rift Valley Province, Kenya, had not been documented since the 1940s, but reports of a serious epidemic in June 1994, followed by discussions with local physicians, suggested that malaria in this highland area was becoming an increasing problem. A USAMRU-Kenya/KEMRI study was initiated in 1997 to gather reliable data to document the scope of the malaria problem and to establish whether the cases were imported by travelers to holoendemic areas, as had previously been assumed, or were due to local transmission. The study data were gathered from a large tea company, with estates at 1,780 to 2,225 meters altitude, and whose health care system serves 100,000 beneficiaries. Records from 1990-1997 showed malaria epidemics occurring almost annually during May-July, with about 50% of beneficiaries diagnosed with malaria each year, 42/100,000/year malaria deaths, and 32% of all deaths being caused by malaria. A questionnaire survey of 244 malaria inpatients in June 1997 revealed that only 8% had traveled to an area with known malaria transmission during the 30 days prior to their diagnosis. Contrary to popular beliefs, it appears that there is efficient local transmission of malaria in this highland area, with increased numbers of cases each year. Given that the data show the local rainfall and temperature to be stable over the last 2 decades, and that the number of travelers to holoendemic areas and the total population of the tea company have not changed, the increasing malaria problem may be due to the failure of currently used drugs to cure infections. This leaves large numbers of gametocyte carriers available to mosquitoes when transmission is favored at mid-year.

ENTOMOLOGICAL STUDIES

The entomology program under this Cooperative Agreement supports the goal of reducing morbidity and mortality of Armed Forces Personnel due to malaria by elucidating interactions between the mosquito vector and the human host, and between the vector and the pathogen; examining variation in local patterns of malaria transmission; and providing critical entomological support data to clinical, immunological, and vaccine studies. Additional studies were conducted on other arthropod vectors of tropical diseases of import to both the U.S. military and the Kenyan Government.

1996 AND 1997 ACCOMPLISHMENTS

After a gap of close to a year, an entomologist was assigned to the KEMRI/USAMRU-Kenya project in mid-1996. Activities for the remainder of that year were concerned with inventorying supplies and equipment on hand, ordering new equipment, assessing the current program, and establishing proposals for new projects. Also during 1996, malaria vector studies were initiated in Kombewa in conjunction with the malaria immunology studies. Using a state-of-the-art Global Positioning System, mapping of villages in the malaria study area was initiated with the goal of better understanding malaria transmission patterns. This extensive undertaking was continued throughout 1997 and should be completed in early 1998.

Also during 1997, a preliminary study of the effectiveness of repellent-treated ground cloths was conducted in Kenya. The cloths, treated with permethrin and intended to be used under and around tentage, proved to be very effective in repelling and killing ticks and other ground-dwelling arthropods. An expanded study, in collaboration with the Walter Reed Army Institute of Research, is being planned for 1998.

A collaborative effort, conducted primarily by entomologists from the Biomedical Sciences Research Centre, KEMRI, with assistance from the USAMRU-Kenya entomologist, was continued in Baringo District, Rift Valley Province, to collect and colonize local sandfly vectors of visceral leishmaniasis. Additionally, colonies of local anopheline mosquito vectors of malaria were established in the laboratory.

ADDITIONAL TROPICAL DISEASE EFFORTS

ENTERIC DISEASES - 1996 AND 1997

Enteric diseases continue to be a major cause of morbidity and mortality in Kenya and a significant infectious disease threat to U.S. forces deployed overseas. In 1995, during an antimalarial drug study in western Kenya, KEMRI and USAMRU-K researchers identified an outbreak of Shigella dysenteriae among the study population. This Shigella species is remarkable in its ability to cause severe dysentery, i.e., bloody diarrhea, in massive epidemics. This discovery led to a renewed interest in the study of enteric diseases in Kenya by USAMRU-Kenya. In 1996 and continuing through 1997, a surveillance effort was established and conducted at a mission hospital in Mumias, Western Province, to determine if the S. dysenteriae epidemic was continuing, to determine the presence of other enteric-disease causing agents and to determine the antibiotic sensitivities of enteric agents present. Stools from 109 nonpregnant adults presenting with bloody diarrhea were analyzed for shigella and salmonella by standard microbiological enteric culture methods. Antibiotic sensitivities were completed using the disc diffusion method. Eighty-three patients (76%) had a shigella dysentery; 54 of these were due to S. dysenteriae type I. Shigella dysenteriae type I isolates were resistant to commonly prescribed drugs. Ninety-nine percent were resistant to tetracycline; 98% to ampicillin; 100% to trimethoprim/sulfamethoxazole; 69% to amoxicillin/clavulanic acid; and 98% to erythromycin. The isolates were sensitive to nalidixic acid (83%), norfloxacin (100%), and gentamicin (100%). Nineteen patients were infected with S. flexneri; 1 with S. sonnei; 1 with S. boydii; 5 with S. dysenteriae that were untypeable; and 3 with Shigella spp. Only 2 patients were infected with Salmonella enteritidis. This survey indicates that the major cause of bloody diarrhea among adults in western Kenya is shigella and that the majority of these isolates are resistant to currently recommended treatment.

In a separate study, antibiotic sensitivities of bacterial enteric pathogens in children with acute diarrhea from a large slum area (Kibera) of Nairobi, Kenya, were examined. Three hundred and seventeen children, ages 2 to 108 months, presenting to the outpatient clinic at Mbagathi District Hospital with acute diarrhea during May and June of 1997 participated in this study. Clinical and epidemiological information, and stool samples or rectal swabs were obtained. Standard microbiological enteric culture methods were used to isolate bacteria. The disc diffusion method was used to determine antibiotic sensitivities. Pathogenic bacteria were found in 179 children; 56 children had more than 1 pathogen identified. Shigella spp was identified in 58 children, Salmonella spp in 13, Campylobacter in 10, and Escherichia coli in 136. The percentage of E. coli, Shigella, and Salmonella isolates sensitive to the following drugs were, respectively: ampicillin- 7, 9, 17%; chloramphenicol- 66, 31, 85%; ciprofloxacin- 99, 100, 100%;

erythromycin- 0, 0, 0%; gentamicin- 97, 96, 85%; nalidixic acid- 98, 98, 85%; norfloxacin- 100, 100, 100%; trimethoprim/sulfamethoxazole- 26, 5, 54%; and tetracycline- 30, 4, 46%. The results indicate a high degree of antimicrobial resistance to the commonly prescribed drugs.

VIRAL DISEASES - 1997

In addition to enteric diseases, there has been a revived interest in the potential for outbreaks of arboviral diseases and viral hemorrhagic fever. The emergence of Yellow Fever (YF) as a public health threat in Kenya resulted in a program of active surveillance for YF in 1993. The sentinel surveillance system quickly proved its value by detecting continuing low-level transmission of YF in endemic regions in Kenya. This monitoring activity has become vital in the identification of high risk population groups to be targeted for YF vaccination. The World Health Organization/U.S. Centers for Disease Control/U.S. Army supported surveillance program in Kenya centered at KEMRI was expanded and updated in 1997 to include other arboviral diseases and viral hemorrhagic fevers. A complete revision of the surveillance program for countrywide implementation is ongoing. This will be complemented with the modernization of the laboratory diagnostic capabilities at KEMRI.

Appendix A ABSTRACTS & PRESENTATIONS

1996

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A phase I trial comparing subcutaneous and intramuscular delivery of Spf66:

Reactogenicity and immunogenicity.

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Placental responses to malaria infections.

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Annual Meeting of the American Society of Cell Biology

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